

Exploring syntrophic relationships in the anaerobic biodegradation of lipids and long chain fatty acids

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Practical knowledge on anaerobic digestion of waste lipids has been improving for several decades, but the microbiology of these processes remains partially undisclosed, with non-cultivated taxonomic groups often detected in anaerobic communities degrading lipids. This work studies the diversity and physiology of anaerobic microorganisms involved in the metabolism of lipids and long chain fatty acids. Anaerobic culturing procedures were applied for the development of enrichment cultures, and combined with next generation sequencing techniques. Enriched microbial communities specialized in the degradation of triolein ($0.3 \text{ mmol}\cdot\text{L}^{-1}$) and oleate ($1 \text{ mmol}\cdot\text{L}^{-1}$) were obtained under methanogenic conditions. Oleate-degrading cultures were also developed in the presence of the external electron acceptors ferric hydroxide ($75 \text{ mmol}\cdot\text{L}^{-1}$) or sulfate ($15 \text{ mmol}\cdot\text{L}^{-1}$). Three mesophilic sludges from different origins were used as inocula.

Bacteria from the genus *Syntrophomonas* were dominant in the different methanogenic and sulfate-reducing enrichments, accounting for 25 % - 49 % of the 16S rRNA gene sequences retrieved. Known microorganisms able to utilize H_2/CO_2 and acetate were also identified, namely *Methanobacterium*, *Methanosarcina* and *Methanoculleus* in methanogenic cultures, and *Desulfovibrio*, *Desulforhabdus* and *Desulfobacca* in sulfate-reducing cultures. The predominance of *Syntrophomonas* in triolein-degrading

enrichment, allied to the fact that genes coding for presumed lipases are present in some *Syntrophomonas*' genomes, suggests a possible role of these bacteria in lipids' hydrolysis. In the iron(III)-reducing culture, *Geobacter* sp. was present at 86 % relative abundance, while *Syntrophomonas* sp. was less abundant (7 %). No oleate degradation was observed when *G. anodireducens*^T (the closest relative to the *Geobacter* sp. present in the enrichment) or *G. bemidjiensis*^T (which possess a long-chain fatty acyl-CoA dehydrogenase coding gene) were incubated with oleate and ferric citrate. This suggests the occurrence of a novel syntrophic relationship, with *Geobacter*-like organisms possibly using the hydrogen and acetate generated from oleate oxidation by *Syntrophomonas*.